



# The Use of Various Concentrations of EM4 (Effective Microorganism-4) with Moringa Leaves (*Moringa oleifera*) as Animal Feed to Reduce Ammonia Levels in Broiler Chicken Manure and Its Implementation in Environmental Pollution Materials

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**Abstract:** This study was motivated by the pungent odor of broiler manure around the farm. Chicken manure that accumulates in chicken coops and accumulates in large quantities will produce harmful gases such as ammonia. Therefore, an alternative solution is needed to manage chicken manure so that the ammonia content is not high. One process that can be used is to provide EM4 and Moringa leaves as animal feed. The purpose of this study was to determine the concentration of EM4 and Moringa leaves that can stabilize ammonia levels in broiler manure. This research is an experimental study using a group randomized design consisting of 4 treatments and each treatment used 1 kg of animal feed and each was given 2 replicates for 4 weeks. With data analysis techniques using the Anova test and the Honest Real Difference test. The design is as follows: A0: 1 kg of animal feed without EM4 and moringa leaves, A1: 1 kg of animal feed with 15 ml EM4 and 9 gr moringa leaves, A2: 1 kg of animal feed with 25 ml EM4 and 9 gr moringa leaves, A3: 1 kg of animal feed with 35 ml EM4 and 9 g moringa leaves. The results of the research conducted obtained that the concentration of EM4 with moringa leaves as the right animal feed is 25 ml EM4 and 9 gr moringa leaves (A2) where the result is the ammonia level of 10 ppm.

**Keywords:** Ammonia; EM4; Moringa leaf

## Introduction

The world of animal husbandry, especially poultry farming, is currently growing rapidly in Indonesia. Many poultry farms are growing due to the demand for chicken meat. The increasing broiler population will certainly have a positive impact, namely the availability of sufficient meat in Indonesia (Suganda et al., 2024). However, the increasing population will also have a negative impact on chickens, humans, and the

environment due to the increasing amount of chicken manure. Chicken manure that accumulates in chicken coops and accumulates in large quantities for days can produce various harmful gases such as ammonia, methane, carbon dioxide (Al-Kerwi et al., 2022). The presence of these harmful gases can cause a decrease and productivity in chickens (Costantino et al., 2020; Apalowo et al., 2024; Pereira et al., 2023). Among the many harmful gases in chicken coops, ammonia and methane are the gases that have the most negative

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impact on chickens, humans and the environment (Tawfik et al., 2023).

Ammonia is one of the compounds that causes the odor of chicken manure due to the decomposition process of bacteria in chicken manure (Chen et al., 2024; Jiang et al., 2023). Based on direct observation conducted in Sumpang Saloe, Cabenge Village, Lilirilau District, Soppeng Regency, workers in their daily work have direct contact with their pets. Direct contact between chicken keepers and pet chickens does not use special personal protective equipment (Mace & Knight, 2024). The odor smelled while at the chicken farm is very pungent and distinctive, so it is estimated that the waste has contaminated the air around the farm and caused a decrease in air quality in the area. Efforts made to control ammonia levels in broiler cages is the provision of EM4 (Effective Microorganism-4) in animal feed. Biotechnology by using EM4 in animal feed as a form of biological waste treatment that utilizes microorganisms attached to a medium to degrade pollutants contained in broiler manure.

EM4 (Effective Microorganism-4) is a collection of microorganism cultures consisting of photosynthetic bacteria, fermentation fungi, lactic acid bacteria and yeast that reduce gross parameters and suppress pathogenic bacteria. Redweik et al. (2020) and Ibrahim (2015), stated that the combination of *Bacillus subtilis* and *Lactobacillus bulgarius* bacteria as probiotics has the potential to reduce ammonia pollution in chicken coops and the application of probiotics can clearly reduce ammonia in chicken manure. Based on the results of Khan et al. (2021) and Rifat et al. (2024), which found that the addition of moringa leaves as a supplement to broiler feed at doses of 3, 6, and 9 g/kg of feed had an effect on reducing ammonia. The decrease in ammonia levels is thought to be influenced by the presence of saponin content in moringa leaves (Leitanthem et al., 2022; Olaoye et al., 2021). However, the weakness of moringa leaves is its high crude fiber content of 12.50% (Saha et al., 2022). According to Sharma et al. (2020) and Senanayake et al. (2023), fermentation is a process that uses microbes as fermenters or inoculants. With fermentation, it will be able to increase crude protein levels and reduce crude fiber levels (Dong et al., 2020; Boonmee et al., 2024).

Based on this exposure, to overcome this weakness, biological processing can be done through the utilization of EM4 (Effective Microorganism-4) in animal feed and combined with Moringa leaves (*Moringa oleifera*). The results of this research will later be associated with learning in Biology subjects with environmental pollution material, students do not have to focus on theoretical studies but students go directly to conduct experiments. In supporting the learning process, a guide or tool is needed in learning in the form of LKS based on

environmental research obtained, so it can be implemented in Biology learning, especially pollution material in accordance with Basic Competencies 4.11 "Propose ideas for solving environmental change problems in the context of environmental problems in their area" and (KI) Provide alternative solutions to environmental pollution problems that occur in the region. Based on the description above, the author will conduct research on "The use of various concentrations of EM4 (Effective Microorganism-4) with Moringa leaves (*Moringa oleifera*) as animal feed to reduce ammonia levels in broiler manure and its implementation in environmental pollution material".

## Method

### Experiment Materials

This type of research is experimental research. The research design used was Randomized Group Design (RAK), with 4 variations of concentration and 2 replications where each treatment used 1 kg of purebred chicken feed that had been mixed with moringa leaves as much as 9 g and EM4 solution with different concentrations of 0, 15, 25, and 35 ml. This research was conducted at Jalan Sumpang Saloe, Cabenge Village, Lilirilau District, Soppeng Regency, from March to May 2023. The design is as follows: A0: Without giving EM4 solution and moringa leaves; A1: Giving 1 kg of animal feed with 15 ml of EM4 solution and 9 g of moringa leaves; A2: Feeding 1 kg of fodder with 25 ml of EM4 solution and 9 g of moringa leaves; A3: Feeding 1 kg of animal feed with 35 ml of EM4 solution and 9 g of moringa leaves. Equipment needed in this review are 4 feed bins or containers for treatment, basin, stone mortar, scales, ammonia test paper. The materials used were farm EM4, Moringa leaves, commercial feed, 0.5 kg bran, 1.5 kg ground corn, 0.5 kg cargin (concentrate), 0.5 kg brown sugar and sufficient water.

### Feed Making

Fresh moringa leaves are picked and collected, separated from the twigs, then dried in the shade and protected from direct sunlight until the moringa leaves are brittle. Then mashed using a blender; Next, 0.5 kg brown sugar is melted by cooking and then prepare a basin to cool the liquid sugar; Prepare 4 containers for treatment. Then, put the moringa flour that was mashed earlier as much as 9 grams with liquid sugar and EM4 solution. Give EM4 with a volume of 0, 15, 25, and 35 ml each and store in the prepared container. Steer and cover for 2 days; Prepare a basin mix 0.5 kg of cargin (concentrate), 0.5 kg of bran, 1.5 kg of ground corn, finished feed and water and mix everything well; After the cargin (concentrate), bran, ground corn, finished feed and water are mixed then put it into each container that

has been treated with 0.5 kg of feed; If it is not sticky then the feed has been created successfully.

#### Data Collection Technique

Data were collected using the experimental method by taking measurements using ammonia test paper. Measurements were taken before treatment to determine the initial ammonia levels of broiler manure and after treatment.

#### Data Analysis Results Technique

To determine the results of using various concentrations of EM4 (Effective Microorganism-4) with Moringa leaves (*Moringa oleifera*) as animal feed on ammonia levels in broiler manure, the results were processed using the ANOVA test. If there is a different effect, then continue with the related Honest Real Difference test.

### Results and Discussion

#### Result

##### Ammonia Measurement in Broiler Manure First Week

Based on the table of observations made, in the first week shows that the A2 treatment given 25 ml EM4 and

9 gr of moringa leaves as animal feed experienced a significant decrease in ammonia levels in broiler manure, namely from 30 to 23 ppm. This is in line with Jiang et al. (2023) who stated that the addition of moringa leaves as a feed additive to lay hens in the last phase as much as 3, 6, and 9 g/kg feed had an effect on reducing litter ammonia concentrations. Compared to treatment A0 which was not given EM4 and moringa leaves, the level of ammonia did not change but increased from 30 to 35 ppm.

**Table 1.** Ammonia measurement of broiler manure in the first week

Treatment	Replay		Amount	Average
	1	2		
A0 (Without EM4 and moringa leaves)	30	35	65	32.50
A1 (15 ml EM4 and 9 g Moringa leaves)	30	24	54	27
A2 (25 ml EM4 and 9 g Moringa leaves)	30	23	53	26.50
A3 (35 ml EM4 and 9 g Moringa leaves)	30	25	55	28.50
Amount	120	107	227	114.50

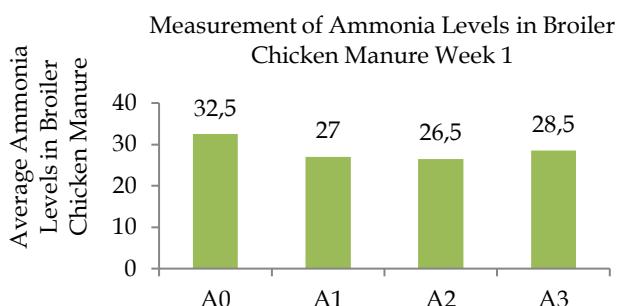
Source: Research Data

**Table 2.** Anova test of ammonia level measurement of broiler races in the first week

Source of Diversity	Free Degree	Sum of Squares	Center Square	F <sub>count</sub>	F <sub>table</sub>	Description
					0.05	0.01
Treatment	3	46.38	15.46	1	9.28	29.46
Group	1	21.13	21.13	1.37	10.13	34.12
Error/Remainder	3	46.38	15.46			
Total	7	113.88				

Notes: tn= No Significant Effect

Based on Table 2 anova test using 5% confidence shows that  $F_{count} 1 < F_{table} 9.28$ . So it can be said that the treatment does not have a significant effect. So, there is no need to continue using the BNJ test.



**Figure 1.** Average ammonia level of broiler manure

#### Second Week

Based on observations made in the second week, it shows that the A2 treatment given 25 ml EM4 and 9 gr

moringa leaves as animal feed showed a significant decrease in broiler ammonia levels from 23 to 22 ppm. Compared to the A1 treatment given 15 ml EM4 and 9 g moringa leaves, the ammonia levels in broiler manure did not change but increased from 23 to 24 ppm.

**Table 3.** Ammonia measurement of broiler manure in the second week

Treatment	Replay		Amount	Average
	1	2		
A0 (Without EM4 and moringa leaves)	35	35	70	35
A1 (15 ml EM4 and 9 g Moringa leaves)	23	24	47	23.50
A2 (25 ml EM4 and 9 g Moringa leaves)	23	22	45	22.5
A3 (35 ml EM4 and 9 g Moringa leaves)	25	25	50	25
Amount	106	106	212	107

Based on Table 4 the anova test using 5% confidence shows that the  $F_{count}$  value is  $199 > F_{table} 9.28$ . So it can be said that the treatment has a significant effect on

reducing ammonia levels in broiler manure where at concentration A2 the ammonia level is 23 to 22 ppm so that it can be continued with the BNJ test.

**Table 4.** Anova test of ammonia level measurement of broiler races in the second week

Source of Diversity	Free Degree	Sum of Squares	Center Square	$F_{count}$	$F_{table}$	0.05	0.01	Description
Treatment	3	199	66.33	199	9.28	29.46		**
Group	1	0.00	0.00	0	10.13	34.12		
Error/Remainder	3	1.00	0.33					
Total	7	200.00						

Notes: \*\*= Significant Effect

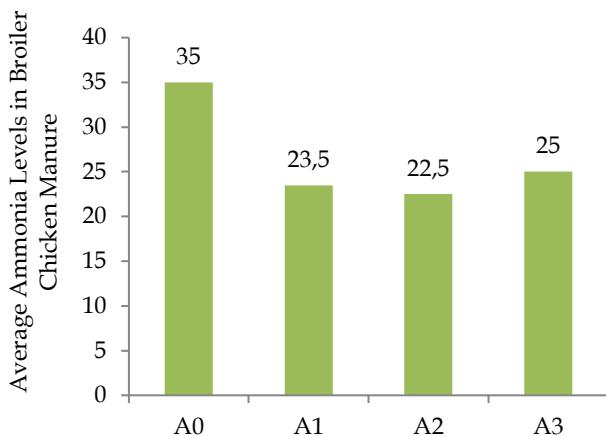
**Table 5.** Average ammonia level of broiler manure in the second week

Treatment	Average	BNJ
A0	35 <sup>c</sup>	
A1	23.50 <sup>ab</sup>	
A2	22.50 <sup>a</sup>	2.41
A3	25 <sup>b</sup>	

Notes: Numbers followed by the same letter are not significantly different at a significant level of  $\alpha 0.05$ .

Based on Table 5 above, it is known that the A0 treatment is significantly different from the A1, A2 and A3 treatments, A2 is different from A3 but not significantly different from A1, and A1 is not significantly different from A3 which can be seen in Figure 2.

Measuring Ammonia Levels in Broiler Chicken Manure Week 2



**Figure 2.** Average ammonia level of broiler manure

Week Three

**Table 6.** Ammonia measurement of broiler manure in the third week

Treatment	Replay		Amount	Average
	1	2		
A0 (Without EM4 and moringa leaves)	35	35	70	35
A1 (15 ml EM4 and 9 g Moringa leaves)	23	20	43	21.50
A2 (25 ml EM4 and 9 g Moringa leaves)	20	15	35	17.50
A3 (35 ml EM4 and 9 g Moringa leaves)	25	25	50	25
Amount	103	95	198	105

Based on the observations made, it shows that in the third week, the A2 treatment given 25 ml EM4 and 9 gr of moringa leaves as animal feed showed a significant decrease in ammonia levels in broiler manure from 20 to 15 ppm. Compared to treatment A0 which was without EM4 with moringa leaves and A3 which was given 35 ml EM4 and 9 gr moringa leaves whose ammonia levels did not change, which remained 35 and 25 ppm.

Based on Table 7, the anova test using 5% confidence shows that the  $F_{count}$  value is  $37.39 > F_{table} 9.28$ . So it can be said that the treatment has a significant effect on reducing ammonia levels of broiler manure where at concentration A2 the ammonia level is 20 to 15 ppm so that it can be continued with the BNJ test.

**Table 7.** Anova test of ammonia level measurement of broilers in the third week

Source of Diversity	Free Degree	Sum of Squares	Center Square	$F_{count}$	$F_{table}$	0.05	0.01	Description
Treatment	3	336.50	112.17	37.39	9.28	29.46		**
Group	1	8.00	8.00	2.67	10.13	34.12		
Error/Remainder	3	9.00	3.00					
Total	7	353.50						

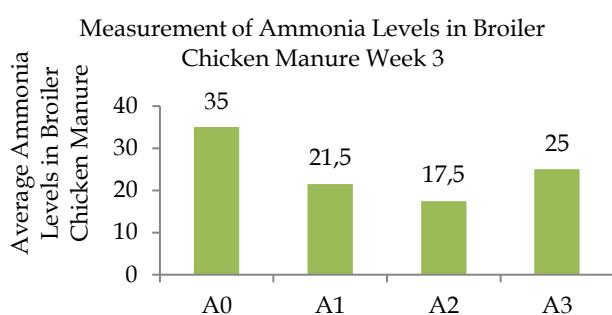
Notes: \*\*= Significant Effect

**Table 8.** Average ammonia level of broiler manure in the third week

Treatment	Average	BNJ
A0	35 <sup>c</sup>	7.23
A1	21.5 <sup>ab</sup>	
A2	17.5 <sup>a</sup>	2.41
A3	25 <sup>b</sup>	

Notes: Numbers followed by the same letter are not significantly different at a significant level of  $\alpha$  0.05.

Based on Table 8 above, it is known that the A0 treatment is significantly different from the A1, A2 and A3 treatments, A2 is different from A3 but not significantly different from A1, and A1 is not significantly different from A3 which can be seen in the following Figure.

**Figure 3.** Average ammonia level of broiler manure**Table 10.** Anova test of ammonia level measurement of broiler breed chickens in the fourth week

Source of Diversity	Free Degree	Sum of Squares	Center Square	F <sub>count</sub>	F <sub>table</sub>		Description
					0.05	0.01	
Treatment	3	559.38	186.46	59.67	9.28	29.46	**
Group	1	28.13	28.13	9.00	10.13	34.12	
Error/Remainder	3	9.38	3.13				
Total	7	596.88					

Notes: \*\*= Significant Effect

Based on Table 10, the anova test using 5% confidence shows that the  $F_{count}$  value is  $59.67 > F_{table}$  9.28. So it can be said that the treatment has a significant effect on reducing ammonia levels of broiler manure where at concentration A2 the ammonia level is 15 to 10 ppm so that it can be continued with the BNJ test.

**Table 11.** Average ammonia level of broiler manure in the fourth week

Treatment	Average	BNJ
A0	35 <sup>c</sup>	
A1	17.50 <sup>ab</sup>	
A2	12.50 <sup>a</sup>	7.38
A3	22.50 <sup>b</sup>	

Notes: Numbers followed by the same letter are not significantly different at a significant level of  $\alpha$  0.05.

#### Fourth Week

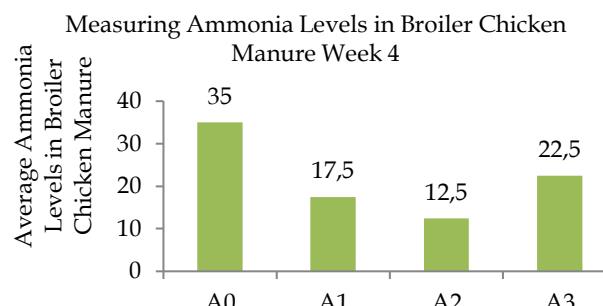
Based on observations made in the fourth week, it shows that the A2 treatment given 25 ml EM4 and 9 gr Moringa leaves showed a significant decrease in broiler ammonia levels from 15 to 10 ppm. This is in accordance with the results of research according to Andersson et al. (2024) and Misto et al. (2024), that  $\text{NH}_3$  levels in cages should not be more than 25 ppm and for humans the threshold level of  $\text{NH}_3$  is 25 ppm for 8-10 hours. Compared to treatment A0, the level of ammonia levels did not change, which remained 35 ppm.

**Table 9.** Ammonia measurement of broiler manure in the fourth week

Treatment	Replay		Amount	Average
	1	2		
A0 (Without EM4 and moringa leaves)	35	35	70	35
A1 (15 ml EM4 and 9 g Moringa leaves)	20	15	35	17.50
A2 (25 ml EM4 and 9 g Moringa leaves)	15	10	25	12.50
A3 (35 ml EM4 and 9 g Moringa leaves)	25	20	45	22.50
Amount	95	80	175	87.50

Source: Research Data

Based On table 11 above, it is known that the A0 treatment is significantly different from the A1, A2 and A3 treatments, A2 is different from A3 but not significantly different from A1, and A1 is not significantly different from A3 which can be seen in the following graph.

**Figure 4.** Average ammonia level of broiler manure

## Discussion

The results of data analysis of ammonia measurements in broiler manure with the addition of EM4 (Effective Microorganism-4) and Moringa leaves (*Moringa oleifera*) as animal feed, namely in experiment A0 without using EM4 and Moringa leaves as animal feed in the first week the ammonia levels of broiler manure were at 30 ppm and after observation in the second, third and fourth weeks the ammonia levels of broiler manure were at 35 ppm, this explains that there is no decrease in ammonia levels in broiler manure but an increase. In the first week, before being given variations in the concentration of EM4 and Moringa leaves as animal feed in broilers with treatments A1, A2 and A3, the ammonia levels were 30 ppm each. After being given, each variation of EM4 concentration of 15, 25, and 35 ml and 9 gr of moringa leaves decreased the ammonia levels of broiler manure, namely A1 = 24, A2 = 23 and A3 = 25. In the second week for treatment variations A1, A2 and A3 given each variation of EM4 concentrations of 15, 25, and 35 ml there was a decrease in ammonia levels of broiler manure, namely A1 = 24, A2 = 22 and A3 = 25.

In the third week, for treatment variations A1, A2 and A3 given each variation of EM4 concentration of 15, 25, and 35 ml, there was a decrease in ammonia levels of broiler manure, namely A1 = 20, A2 = 12 and A3 = 25. While in the fourth week, for treatment variations A1, A2 and A3 given each variation of EM4 concentrations of 15, 25, and 35 ml there was a decrease in ammonia levels of broiler manure, namely A1 = 15, A2 = 10 and A3 = 20. In addition to being based on these observations, the results of analysis of variance (ANOVA) also strengthen the impact of using EM4 and Moringa leaves as animal feed on reducing ammonia levels in broiler manure. The ANOVA results explained the very large effect on reducing ammonia levels in broiler manure in weeks 2, 3 and 4 where  $F_{\text{count}} > F_{\text{table}} (199)$  at the 0.05 level but did not have a significant effect on week 1 where  $F_{\text{count}} < F_{\text{table}} (1)$  at the 0.05 level.

The next test using the 5% BNJ test is intended to determine the best treatment difference for each treatment given the BNJ test. The treatment that differs significantly in this review is treatment A2 with an average ammonia level in broiler manure of 12.5 ppm. This is because the concentration of EM4 and Moringa leaves as animal feed is given in sufficient and appropriate amounts so that it can reduce ammonia levels in broiler manure (Amad & Zentek, 2023; Su & Chen, 2020; Donkor et al., 2013). Based on the results of the analysis of research data obtained in the A2 treatment, there is a significant effect, namely a decrease in ammonia levels in broiler manure where the results are before being treated, ammonia levels in broiler manure are 30 ppm. After being given the treatment, in

the first week for the second replicate the ammonia levels in broiler manure were 23 ppm, the second week 22 ppm, the third week 12 ppm and the fourth week 10 ppm. These results are also in line with Gržinić et al. (2023), Chaachouay & Zidane (2024), Khmaissa et al. (2024), and Hafez & Attia (2020) research on livestock herbal medicine on a laboratory scale, which found that poultry given livestock herbal medicine had a low mortality rate (below 10%), healthier chickens, more environmentally friendly (ammonia odor from manure is reduced), less abdominal fat and more efficient and economical use of feed (Zhang et al., 2023).

## Implementation of Research Results as Teaching Materials in SMA Class X

In the 2013 curriculum, environmental pollution learning materials in class X include the basic concepts of analyzing data on environmental changes and their causes, as well as the impact of these life changes and proposing ideas for solving environmental change problems in the context of environmental problems in their area. The skill competency is to provide alternative solutions to environmental pollution problems that occur in the region, and in accordance with KD 4.11 "Propose ideas for solving environmental change problems in accordance with the context of environmental problems in the region" KI provides alternative solutions to environmental pollution problems that occur in the region (Abalansa et al., 2021). Lesson plans are made as learning tools used by biology teachers and practicum worksheets are made to facilitate the student learning process (Berg & Plessis, 2023; Koberstein-Schwarz & Meisert, 2023). In the learning process the teacher will provide practicum worksheets to students to make it easier for students to know how the practicum works (Widestra et al., 2020).

Based on the research activities carried out, students are directed to find problems that occur around them, especially in environmental pollution, students are required to be able to solve the problems they find (Awewomom et al., 2024; Kumar et al., 2021). Students will carry out practicum in groups by following the procedures contained in the practicum worksheet that has been given (Jenssen & Haara, 2024; Wola et al., 2023). After that, the results of the practicum are presented in front of the class, in the presentation students are expected to get an overview or knowledge in solving problems about environmental problems (Faize & Akhtar, 2020).

## Conclusion

From the results of this study it can be concluded that giving the concentration of EM4 (Effective Microorganism-4) with Moringa leaves (*Moringa*

oleifera) as animal feed with a dose of 25 ml EM4 and 9 g Moringa leaves showed a decrease in ammonia levels in broiler manure which was the best compared to other groups. However, the treatment that was not given EM4 and Moringa leaves actually increased ammonia levels. The use of EM4 (Effective Microorganism-4) and Moringa oleifera leaves as animal feed to reduce ammonia levels in broiler manure can be used as teaching material in the Biology learning process on Environmental Pollution material in the form of a Learning Implementation Plan (RPP).

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### Author Contributions

A.B.R. and D.: conceptualized the research idea, research method, and analyzed the data; R.I. and A.B.R.: guided the writing of the review and editing, supervised and validated the instruments used in the research.

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### Conflicts of Interest

The authors declare no conflict of interest.

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